INSTITUTO POLITÉCNICO DE BRAGANÇA

# Dephosphorization of Iron Ore through Bioleaching

**Kanan Mammadov**

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## ABSTRACT

Phosphorus, as phosphate, is frequently found as a constituent of many of the world iron resources. Phosphorus is an extremely harmful element found in iron ore used as a raw material in the steelmaking process because it will affect the quality of iron and steel products. Allowable phosphorus concentration in high quality steel is usually less than 0.08%. Dephosphorization of iron ore has been studied for a long time. Although there are described physical beneficiation and chemical leaching processes, involving inorganic acids, to reduce phosphorus content of iron ores, these processes have several limitations such as poor recovery, require high energy quantity, capital costs and cause environmental pollution.

Use of microorganisms in leaching of mineral ores is gaining importance due to the implementation of stricter environmental rules. Microbes convert metal compounds into their water soluble forms and are biocatalysts of leaching processes. Biotechnology is considered as an eco-friendly, promising, and revolutionary solution to these problems. Microorganisms play a critical role in natural phosphorus cycle and the process of phosphate solubilization by microorganisms has been known for many years.

This study was performed to analyze the possibility of using bioleaching as a process for the dephosphorization of an iron ore from Northeast of Portugal. For bioleaching, *Acidithiobacillus ferrooxidans* bacterium were used. For this study two experiments were done with different conditions, which lasts 6 weeks for first experiment and 5 weeks for second experiment. From the result of these preliminary studies, it was observed that for first experiment 6.2 % and for second experiment 3.7 % of phosphorus was removed from iron ore.



**Keyword:** Iron ore, phosphorus, bioleaching, *Acidithiobacillus ferrooxidans*

# Resumo

O fósforo é um dos elementos mais nocivos na produção de aço: a sua presença em teores elevados torna o aço quebradiço e com fraturas. O teor máximo de fósforo permitido é de aproximadamente 0,08 %. Muitas das atuais fontes mundiais de minério de ferro contêm teores de fósforo superiores a 1 % (m/m), o que as torna inadequadas para a produção de ferro e de aço sem um pré-tratamento de desfosforação. No entanto, as técnicas de desfosforação mais utilizadas (nomeadamente os processos térmicos ou os que envolvem a utilização de ácidos ou bases fortes) têm sido recentemente criticadas, sobretudo pelo seu elevado custo e também por possíveis efeitos prejudiciais do ponto de vista ambiental. Considerando as preocupações ambientais atuais relativas ao processo de separação de fósforo do minério de ferro, torna-se particularmente relevante o estudo de outros processos alternativos que resultem na produção de ferro com teores baixos em fósforo. No processo de biolixiviação os microrganismos, como consequência do seu metabolismo, produzem subprodutos químicos que atacam o minério e permitem a dissolução e remoção seletiva da ganga.

Este trabalho teve como objetivo a realização de ensaios preliminares para avaliação da ação do microrganismo *Acidithiobacillus ferrooxidans* na desfosforação de um minério de ferro proveniente do Nordeste de Portugal. Realizaram-se dois ensaios de biolixiviação à temperatura de 28°C, razão sólidos/líquidos de 1/10, tendo o primeiro decorrido durante 42 dias e o segundo durante 28 dias. Ao fim deste período de tempo, a percentagem de fósforo removida no primeiro ensaio foi 6,2% e no segundo foi 3,7%.

**Palavras-chave:** minério de ferro, fósforo, biolixiviação, *Acidithiobacillus ferrooxidans*

# Table of Contents

# List of Figures



# List of Graphics

# List of Tables

# 1. INTRODUCTION

Iron is one of the most essential, naturally occurring metals on earth. It is the second abundant metal compromising a percentage of 5 of the earth's crust. This crucial metal rarely exists in pure form as it has great affinity to react with oxygen and form iron oxide. Iron can exist in several hundred minerals but only few are the major primary sources of iron ore minerals.

Dephosphorization of high-phosphorus iron ore has been studied worldwide for several decades. Many efforts have been devoted to physical beneficiation methods of high-phosphorus iron ores. Some chemical leaching processes have been reported to reduce the phosphorus content of iron ores, the relatively high cost in mineral acids and anticorrosive equipment as well as the pollution problems incurred by the production of mineral acids make them unfeasible alternatives.

Biotechnology could be a cost-effective and environment friendly way to overcome these problems. Microorganisms play a critical role in natural phosphorus cycle and phosphate solubilization by microorganisms has been known for many years. Under starvation conditions, microorganisms can utilize phosphorus from mineral sources. Some works have dealt with the use of fungal metabolites and heterotrophic bacteria to attain dephosphorization.

## 1.1. BACKGROUND AND MOTIVATION

The content of phosphorus in iron ore is very important to the production of steel of high quality. Dephosphorization of high-phosphorus iron ore is an unsolved problem worldwide so far. Biotechnology could be a cost-effective and environment-friendly way to solve this problem.

In this work, the capability of dephosphorization of an iron ore from a mine from Northeast of Portugal, using *Acidithiobacillus ferrooxidans* is studied by bioleaching.

The main objective of this work is to reduce phosphorus content in iron ore using bioleaching. For this purpose preliminary studies of bioleaching were done with iron ore from a mine in Portugal. We intend to study the effect of some parameters such as pH, temperature, particle size, inoculum concentration and solid/liquid ratio in the yield of phosphorus removal that are important factors in bioleaching processes.

## 1.2. MAIN OBJECTIVES

The main purpose of this work is to develop a bioleaching process to remove phosphorus from an iron ore using the degradation ability of microorganisms. With this purpose two preliminary studies were done to analyse the yield of phosphorus removal from the iron ore. Iron ore received from a mine in Portugal has 0.67% of phosphorus and it is intended to decrease this value to a level that allows the use of iron in the production of steel.

The experiments were done at 28°C, using *Acidithiobacillus ferrooxidans* as inoculums, the ratio solid/liquid is 1/10. Almost 90% of the particles has size between 75 and 425 µm.

## 1.3. OUTLINE OF THE REPORT

The first Chapter of this thesis work is an introduction to the work with brief information about hydrometallurgy, dephosphorization and bioleaching process. Chapter 2 exposes the process itself, gives information about what is iron ore and process benefits and drawbacks. Research strategy in Chapter 3 presents the materials, describes the methodology and details the analysis parameters adopted. Chapter 4 presents obtained results with discussion. Chapter 5 covers all analysis and conclusions of the work.

# 2. LITERATURE REVIEW

## 2. 1. IRON ORE

Iron is one of the most essential, naturally occurring metals on earth. It is the second abundant metal compromising a percentage of 5 of the earth's crust. This crucial metal rarely exists in pure form as it has great affinity to react with Oxygen and form Iron oxide. Iron can exist in several hundred minerals but only few are the major primary sources of Iron ore minerals. These comprise magnetite ($Fe_3O_4$), hematite ($Fe_2O_3$), goethite ($Fe_2O_3H_2O$), siderite ($FeCO_3$), pyrite ($FeS_2$). The geological processes behind the formation of Iron ore are generally by direct sedimentation, igneous activity and weathering (http://www.gsa.org.au/) **[1]**.

**Magnetite ($Fe_3O_4$):** The name has its origin as large deposits were discovered from the district Magnesia, Asia. It contains both reduced (Ferrous) and Oxidized (Ferric) iron species and so, it is also termed as Iron $^{II, III}$ oxide. The equation presented below as Figure 1 gives a clear idea on the composition of magnetite **[2]**.

$$\underset{\text{Ferrous oxide}}{FeO} + \underset{\text{Ferric oxide}}{Fe_2O_3} \rightarrow \underset{\text{Magnetite}}{Fe_3O_4}$$

Figure 1. Synthesis of magnetite

**Hematite ($Fe_2O_3$):** It is available in abundance in soils and sediments. Its crystal structure elucidates that the most common substituent is aluminum. The applications of hematite include catalyst and photocatalyst functions. Its dielectric properties are suitable for photoelectrochemical characterization to employ its application in solar photoelectrochemical derived use **[3]**.

**Siderite -** has chemical composition of $FeCO_3$– iron carbonate and it contains 48.2% of iron. Siderite ore is named a sperry iron-stone, or siderite. If significant amounts of clay impurities are present it can be called clay ironstone. Siderites are widespread far fewer than other ores. Characterized by low maintenance of iron. Under action of moisture and oxygen of atmosphere siderites can pass to limonite, because the oxide of iron (II) in the molecule of $FeO.CO_2$ oxidized

and takes in moisture. Therefore there are deposits in those epiphytes of ore those are limonite and also lower native siderites.

**Pyrite:** Iron sulphide $FeS_2$ occurs in nature in the form of the mineral pyrite, or iron pyrites. It contains 46.6% iron. It is used in the chemical industry, where it is combusted to sulphur separation. Iron is oxidized in the form of pyrite cinders used in producing the agglomerate.

**Goethite ($Fe_2O_3.H_2O$):** The goethite are secondary Fe minerals precipitated from groundwater contaminated by mine leachate **[4]**.Also goethite, α -FeO(OH), is one of the most widespread forms of iron oxides in terrestrial soils, sediments and ore deposits as well as a common weathering product in rocks of all types. It was reported by Hexiong Yanget and co-workers that they transforms to hematite ($\alpha$-$Fe_2O_3$) between 453 and 543 K through dehydrogenation and has been used extensively in the preparation of magnetite ($\Upsilon$-$Fe_2O_3$) in magnetic storage media **[5].**

**APATITE [$Ca_5(PO_4)(F, Cl, OH)$]**: Apatite iron oxide ores are the Sweden's main iron source. Sweden stands by far the largest iron producer in Europe. The type of apatite iron oxide produced is 'Kiruna Type'. The most frequent and important of the class of phosphates is Apatite **[6]**. The others are also worth to be mentioned. They can be seen in the Table 1 below.

Apatite mainly exists as disseminated grains in the ore or form schliren or vienlets. These slightly coarser in grain sized patches-stringers-schliren occur in combinations with biotite, magnetite and calcite. The apatite is a fluorapatite with 3.75 % F and 0.06 % Cl on average. There is no significant variation in composition for apatite in the tailings and samples of ore **[7]**.

Table 1. Main Phosphorus minerals in iron ore **[8]**

| **Mineral** | **Chemical formula** |
|---|---|
| Apatite | $Ca_5(PO_4)(F, Cl, OH)$ |
| Wavellite | $Al_3(PO_4)_2(OH)_3 \times 5(H_2O)$ |
| Senegalite | $Al_2(PO_4)(OH)_3 \times H_2O$ |
| Turquoise | $CuAl_6(PO_4)_4(OH)_8 \times 5(H_2O)$ |
| Strengite | $Fe^{3+}(PO_4) \times 2(H_2O)$ |
| Rockbridgeite | $Fe^{2+}\ 0.75Mn^{2+}0.25Fe^{3+}4(PO_4)_3(OH)_5$ |
| Frondelite | $Mn^{2+}Fe^{3+}4(PO_4)_3(OH)_5$ |
| Gorceixite | $BaAl_3(PO_4)_2(OH)_5 \times H_2O$ |
| Barrandite | $(Al,Fe)PO_4 \times 2(H_2O)$ |
| Variscite | $Al(PO_4) \times 2(H_2O)$ |

## 2.2. HYDROMETALLURGY

It all has begun in the good olden times of alchemists, where the transmutation of base metals into gold was their primary importance. The best example is a piece of iron dipped in an Arab alchemist by name Jabir Ibn Hayyan (720 – 813 AD) discovered aqua regia, which is considered as the gold standard mark for the beginning of hydrometallurgical era. The aqua regia (royal water) is a mixture of HCl and $HNO_3$, in a molar ratio of 3/1. It is still in use as a source for refining gold. The Table 2 is reproduced from **[9]** and gives detailed historical developments in hydrometallurgy, chronologically.

Table 2. Historical developments in hydrometallurgy.

| | |
|---|---|
| **Early period** | |
| 7th Century | Transmutation of iron into copper by alchemists |
| 8th Century | Discovery of aqua regia (royal water). Only solvent for gold used even today for gold refining. |
| 16th Century | Heap leaching of copper containing pyrite, and precipitation of copper from the solutions by iron. |
| 18th Century | Production of potash for soap and glass industries by leaching ashes left after burning wood. |
| **Modern era** | |
| 1887 | The invention of the cyanidation process, i.e., dissolution of gold from ores by a dilute sodium cyanide solution and the precipitation of gold from the solutions by zinc. The invention of Bayer's process: precipitation of crystalline $Al(OH)_3$ from sodium aluminate solution by seeding. |
| 1912 | Recovery of copper from leach solution in Chile by electrolysis. |
| 1916 | The use of ammonium hydroxide for leaching native copper ore in Lake Superior District, and for malachite-azurite ore in Alaska. Development of the hydrometallurgical electrowinning zinc process at Trail and Anaconda. The recovery of cadmium as a by-product of the zinc hydrometallurgical process. |
| 1924 | Caron process for ammonia leaching of metallic nickel produced by reduction of laterites. |
| 1927 | Henglein process for pressure leaching of ZnS at high temperature in the presence of oxygen. |
| 1930 | Sullivan process for ambient leaching of copper sulphides by ferric chloride solution. |
| **Development during World War II** | |
| 1940s | Development of the uranium technology. Introduction of sodium carbonate as a leaching agent for uranium, the widespread use of ion exchange and solvent extraction for uranium recovery, and the separation of the lanthanides by ion exchange. |
| **Recent advances** | |
| 1950s | The application of pressure hydrometallurgy for leaching nickel sulphide ores. |
| 1960s | Discovery of the role played by microorganisms in leaching processes and the widespread use of heap and in situ leaching for extracting copper from low-grade material. |
| 1970s | Discovery of galvanic action in leaching sulphide minerals. |
| 1980s | The hydrometallurgy of gold greatly advanced: widespread application of activated carbon technology, and aqueous oxidation of gold refractory ores. |

## 2.3. PHOSPHOROUS

Phosphorus was accidentally discovered in the urine in 1669 by Hennig Brandt. The discovery came into lights when it was finding for a liquid that can transmute silver into gold. He named this white waxy substance as cold fire. Succeeding too many researchers trying to find out the secret process of phosphorus production, ultimately it is in between 1743 and 1746, Andreass Marggraff found this new substance in plant seeds. He also explained that phosphorus enters human system through plants. This new substance shined well the dark, inflamed on slight initiation, burned brilliantly with high flame, and wasted unless kept under water **[10].**

## 2.4. APPLICATIONS

Phosphorus (P) is essential to all known life forms because it is a key element in many physiological and biochemical processes. A component of every cell in all living organisms, phosphorus is indispensable and cannot be replaced by any other element. Phosphorus occurs in complex DNA and RNA structures which hold and translate genetic information and so control all living processes in plants, animals and man. It is an essential component of the energy transport system in all cells. The element phosphorus does not occur by itself in nature. It is always combined with other elements to form phosphates. Phosphates can be very complex and more than one form of phosphate will be found in soils, water, plants, animals and man. At present there are three main uses of phosphorus. In Western Europe some 79% is used to make fertilizer for use in agriculture for food production, around 11% is used to make feed grade additives for animal feeding stuffs, and approximately 7% is used to make detergents. The remainder is used in specialty products as diverse as additives for human food and metal surface treatments to delay corrosion.

Until comparatively recent times the growth of plants and animals, and hence the productivity of agriculture, was limited by a lack of phosphorus since only small amounts are released annually from rocks and soil minerals by weathering. As farmers began to use fertilizers in the 19th century, levels of plant available phosphorus in many soils were still very low. This meant that there was little response to other nutrients, especially nitrogen, until phosphorus was applied, i.e. phosphorus was the limiting nutrient to crop growth.

The chemistry of phosphorus in the soil is complex because the phosphorus is associated with many different compounds to which it is bound with a range of bonding energies or strengths. When phosphates fertilizers are added to soil, only a fraction of the phosphorus is taken up immediately by the plant root. The remainder becomes adsorbed (attached to the surface) to soil

particles. Where the attachment is weak, the phosphorus can transfer back into the soil solution. After the initial adsorption, further reactions lead to absorption (assimilation), which means that the bond is stronger and the phosphorus becomes less readily available. The speed of these reactions and therefore the speed at which a deficiency in phosphorus becomes apparent depends very much on the type and size of the mineral particles, the presence of other elements such as aluminum, iron and calcium, soil acidity and organic matter. Organic phosphorus in soil can be associated either with soil organic matter (humus) or recently added organic debris coming from plants or animals. These organic molecules cannot be used directly by plants. They have to be broken down by soil microbes to release inorganic phosphate ions which can be taken up by plant roots or enter into the same reactions as other fertilizer phosphate ions. Phosphorus in the soil solution is either taken up by plant roots or goes into a readily available pool where it is held weakly. Past concepts about the behaviour of phosphorus in soil suggested that phosphorus added in fertilizers reacted with other soil constituents and became permanently fixed in soil and therefore unavailable to future crops. It is now known that this is not always the case for many soils. When phosphorus is added to the soil it becomes associated with various soil elements. Although only a small proportion of the phosphorus in each application of fertilizer is held weakly, the amount increases as the number of fertilizer applications increase. This explains why the availability of phosphorus has actually increased in soils which have been cultivated and fertilized over many years **[11]**.

## 2.5. PHOSPHOROUS REMOVAL

It is a natural fact that phosphorous is usually integrated in the crystal lattice of iron oxides or in gangue minerals. The obtaining ability of steel from these resources has become quite difficult due to phosphorus presence. Therefore only low phosphorus ores (< 0.08 wt %) are targeted for extraction leaving behind high profile phosphorus ores. The other most important aspect of this issue is that the steel produced or extracted from such high phosphorus ores will be brittle of standards and not good for use in daily needs. Hence, there is a need for dephosphorization. As the need for steel increases, the need to use latest technologies for dephosphorization of iron ores becomes a necessity. Many traditional technologies have become expensive, required more man power and not environmental friendly. In the process for search of new technologies without these drawbacks, bacterial leaching was found to be more effective and a revolutionary solution **[12]**.

## 2.6. EXTRACTION

There are three possible processes exist for extraction of phosphorus from iron ore resources. They are physical, chemical and biological. Each one of them is explained individually in the following paragraphs.

1. **Chemical processes**: Acid leaching has been considered as one of the most effective techniques involved to remove phosphorus from fine particle sinter feed, contrasting the requirements in flotation. Two good case studies elucidate the research results obtained with different acids.

a. In a study by Pereira et.al **[8],** the extraction protocol used either acid or basic media. Samples of particle size smaller than0.075mm having a phosphorus percentage of 1.12 % were analyzed for test. The phosphorus distributions accounted for 22.3 % in apatite, 67.9 % in iron matrix and 9.8 % in silicates. Leaching media used were sodium, hydrochloric acid, sulphuric acid and nitric acid. The results of this study have shown that phosphorus removal by alkaline leaching is effective with apatite and silicates but rendered ineffective with iron matrix. Acids tend to be the most effective compared to basic media, and of all sulphuric acid was the most effective acid to remove phosphorus from iron ore.

b. In another study by JIN, Y. et al **[13]** nitric acid was used to remove phosphorus from iron ore of Kiruna region, Sweden. The results obtained have shown that the removal of phosphorus reached 95 %. The final content achieved the required specification for production of steel.

2. **Physical processes**:

**a. Selective agglomeration**: Acid leaching using $H_2SO_4$ has not given results to satisfaction and so, selective agglomeration technique was preferred by researchers, Sparks and Sirianni in 1974. After jigging, the assays of selective agglomeration were applied to concentrates. The source of the whole process reproduced from **[11]** is shown in Figure 2.

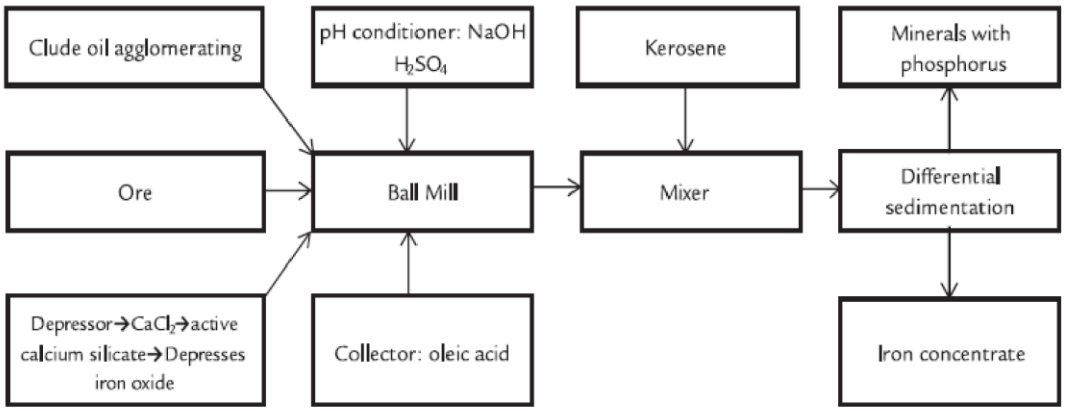



Figure 2. Selective agglomeration work flow

**b. Flotation**: The reduction of phosphorus in iron ore through anionic flotation method, where the fatty acid is used as a collector and sodium silicate as a depressant of iron oxides. In this process, the main phosphorus bearing mineral is usually apatite **[14]**.

**c. Thermal process- Sintering**: The phenomenon that transforms crystalline and or amorphous particles into rigid body due to diffusion is sintering. It is a thermally activated phenomenon. The process can have sintering states like liquid, solid and viscous in case of ceramic reactions. The flow chart as Figure 3 represents the steps involved in thermal process-sintering technique **[13]**.

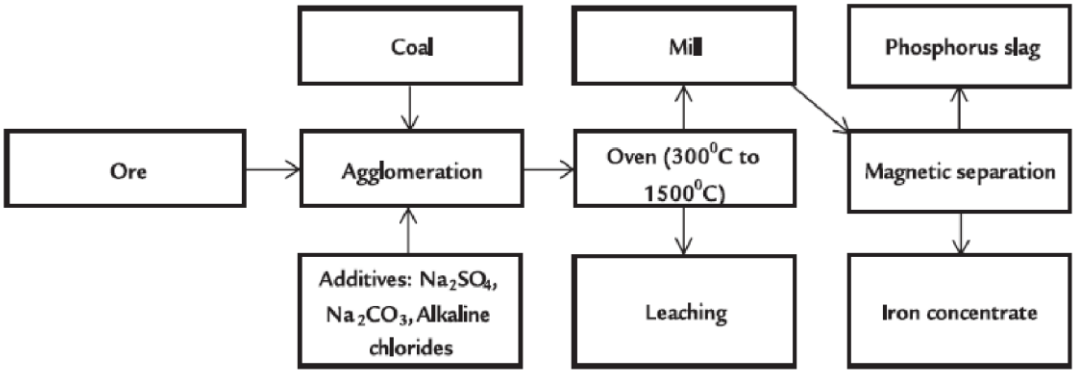



Figure 3. The steps involved in thermal process-sintering technique **[13].**

3. **Biological processes**: The biological involvement and basis for phosphorus removal by microorganisms is that when in limited phosphorus conditions the microbes need to extract the specific mineral from sources for their growth needs. This principle helps in removal of phosphorus mineral for iron ores with many oxidizing bacteria and filamentous Fungi. Therefore, bioleaching process has taken an edge over physical and chemical techniques for its inexpensiveness and less man power and more effectiveness. The main methods include heap leaching, dump leaching and leaching in stirred tanks, pressure leaching and in sit leaching **[15]**.

## 2.7. BIOLEACHING

There are several definitions for Bioleaching process, whereas in general it is dissolution of metals from their mineral resources by the action of naturally occurring specific microorganisms. This is a latest technology from the field of bio-hydrometallurgy under the discipline Biotechnology. It has been advancing at a rapid pace when compared to the other related processes like bioremediation, biosorption and bioaccumulation **[16]**. The reason can be its main application in extracting metals from their ores.

Bioleaching process dates back to sixteenth century in the recovery of copper from mine waters. The Rio Tinto mines in the country of Spain are considered to be the beginners frame for bio-hydrometallurgical processes. The establishment of bioleaching techniques in these mines were begun only in 1890's, after the ban of open air ore roasting due to its atmospheric sulphur emissions in Portugal in 1878. There exist two mechanisms of bioleaching processes, termed as direct and indirect **[17]**.

**Direct bacterial leaching**: In the direct process, many enzymatic catalysed steps favour the oxidation of mineral sulphide to sulphate, when the bacterial cell comes in physical contact with the mineral surface. The steps below shows the reactions involving pyrite oxidized to iron (III) sulphate.

$$4FeS_2 + 14O_2 + 4H_2O = 4FeSO_4 + 4H_2SO_4 \quad (2)$$

$$4FeSO_4 + O_2 + 2H_2SO_4 = 2Fe_2(SO_4)_3 + 2H_2O \quad (3)$$

In one single step it is represented as:

$$4FeS_2 + 15O_2 + 2H_2O = 2Fe_2(SO_4)_3 + 2H_2SO_4 \quad (4)$$

**Indirect bacterial leaching:** In this process, the bacteria need not be in physical contact with the mineral surface. They only have a catalytic function in reoxidation of ferrous iron, which otherwise will take place at a much slower pace. Instead of physical contact, they produce a lixiviant that can chemically oxidize the sulphide mineral. The lixiviant is a Ferric ion in acid solutions **[17]**. Metal solubilisation can be seen in the equation below:

$$MeS + Fe_2(SO_4)_3 = MeSO_4 + 2FeSO_4 + S \quad (5)$$

The bacterial oxidation of ferrous iron is $10^5$ to $10^6$ times greater than the normal chemical oxidation in acidic pH ranges of 2 to 3 **[18]**. The processes of bioleaching depend on several factors like pH, nutrients required for microbial growth, oxygen and carbon-dioxide levels, mineral substrates, temperature, heavy metals, surfactants and organic extractants **[17]**.

## 2.7.1. BIOLEACHING OF IRON ORES BY BACTERIA AND FUNGI

The major gangue minerals of iron ores are alumina, silica, sulphur, and phosphorous. These are well known to show effects on the reducibility of iron oxides, coke rate consumption, blast furnace operation and the productivity for steel making. The hot topic of today is the leaching advancements in the case of iron ores, realizing its purpose in everyday life. The most technologically effective and economically beneficiary process is using heterotrophic bacteria and fungi for removal of these gangue mineral impurities from iron ores.

From the discovery of *Thiobacillus ferrooxidans* in 1951 until the discovery of *Leptospirillum ferrooxidans* in 1972, it was the only acidophilic iron oxidizing bacterium. In 2000, Kelly and Wood re-described these species and categorized *Thiobacillus thiooxidans, Thiobacilluscaldus and Thiobacillus ferrooxidans* to a new genus, *Acidithiobacillus. Leptospirillum ferrooxidans* and *Thiobacillus ferrooxidans* are equally significant in bioleaching. The two mixed

culture shows oxidization of pyrite faster. This is due to affinity to attach to sulphide minerals and also high affinity for ferrous ion and low sensitivity to inhibition by ferric iron **[19]**.

The optimization of process parameters of biohydrometallurgical leaching of iron sulphide ore in an aerobic batch system was done by **[20]**. The results yielded optimized time as 172 hours, pH 2.5 and temperature 35°C, glucose and nitrogen concentration for maximum bioleaching was 3.0 and 0.3 % respectively, shaking speed of 60 rpm. 20 % (v/v) and 7 days old culture was used for the study and bacteria tolerated 35 $kg/m^3$ of initial ferrous iron concentration.

Research studies on understanding the success in leaching of iron ore by fungal strains such as *Aspergillus fumigatus*, *Penicilliumcitrinum*, and *Aspergillus flavus* resulted in 7%, 6%, and 17% removal of alumina, and 8%, 4%, and 16% removal of silica, respectively. The bacterial strains such as *Bacillus polymyxa*, *Bacillus sphaericus*, and *Pseudomonas putida* have shown silica removal percentage of 10.6%, 5.3%, and 20%, respectively. The results of this research has shown that the fungal strain *Aspergillus flavus* and the bacterial strain *Pseudomonas putida* were most efficient among all used in this study. These microorganisms displayed an increase in iron content of more than 3% in 10 days of leaching process **[21]**.

## 2.7.2. PROS AND CONS OF BIOLEACHING

**PROS**:

a. Flexible and can be used for single or mixed minerals.
b. Comparatively more economical and simpler process than the conventional processes.
c. It can be applied at larger scales from simple to complex bioreactors.
d. High temperatures and pressures are not essential.
e. More environmental friendly.
f. $SO_2$ emissions are not produced **[22].**

**CONS**:

a. A very slow process
b. Surface water contamination by $H^+$ ions or sulphuric acid leakages, leading to environmental damage.
c. Biosafety failures are a possible threat **[22].**

# 3. EXPERIMENTAL DESCRIPTION

## 3.1. CHEMICALS

For the laboratory experiments was used an iron ore from a mine in the region of Northeast of Portugal. The following chemical reagents were used for the preparation of the medium used for the bioleaching experiments:

For the analysis of phosphorus in the bioleaching solution the following reagents were used:

Table 3. Chemicals used for the study

| Reagent | Chemical Formula | Supplier | Purity |
|---|---|---|---|
| Ammonium sulphate | $(NH_4)_2SO_4$ | Pronalab | PA |
| Magnesium chloride 6 hydrate | $MgCl_2$ x $6H_2O$ | Panreac | PA |
| Dehydrate calcium chloride | $CaCl_2$ x $2H_2O$ | Merck | |
| Monopotassium phosphate | $KH_2PO_4$ | Projecto | |
| Iron (II) sulfate 7 hydrate | $FeSO_4$ x $7H_2O$ | Panreac | |
| Potassium antimony tartrate | $K(SbO)C_4H_4O_6$ x 1/2 $H_2O$ | Sigma Aldrich | |
| Sulfuric acid | $H_2SO_4$ | Sigma Aldrich | 95-97% |
| Ascorbic acid | $C_6H_8O_6$ | José M.Vaz Pereira | |
| Ammonium molybdate | $(NH_4)_6Mo_7O_{24}$ x $4H_2O$ | Panreac | PA |
| Nitric acid | $HNO_3$ | Riedel-de Haen | 65% |
| Iron standard solution | $Fe(NO_3)_3$ | Eisen-Standardlosung | 0.5 mol/L in nitric acid |

**Microorganisms**

*Acidithiobacillus ferrooxidans* was purchased from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen,German - Collection of Microorganisms and Cell Cultures GmbH). *Acidithiobacillus ferrooxidans* is an autotrophic, acidophilic bacterium. Mobile strains have one flagellum and fimbriae.

According to information of the supplier, *Acidithiobacillus ferrooxidans* thrives at an optimum temperature of 30ºC, being the standard incubation time of 3-7 days.

## 3.2. MATERIALS AND EQUIPMENT

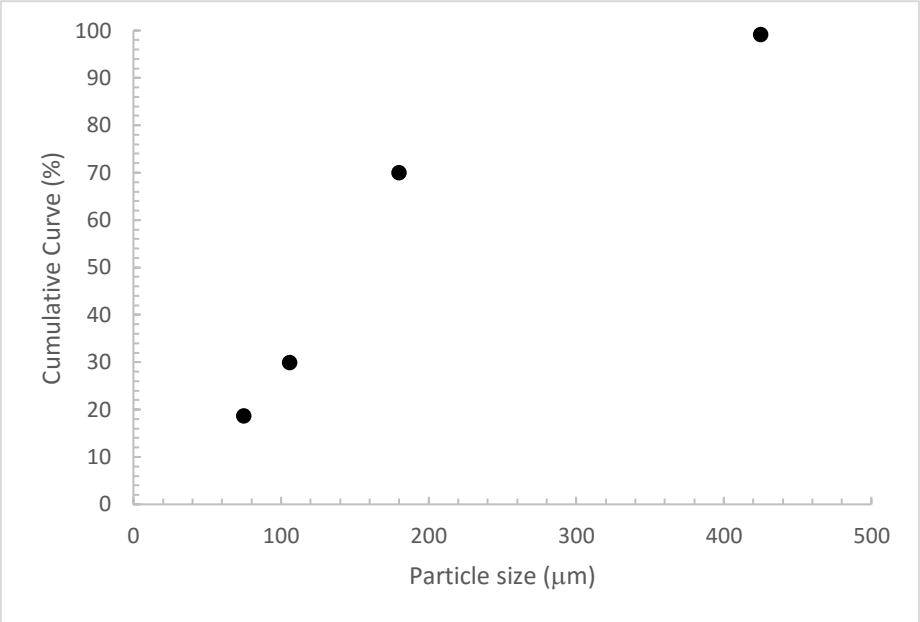
For the experimental part the following materials were used: 40,80,120,200 mesh sieve (determination of the distribution size of the particles) , 100 mL beaker, 250 mL Erlenmayer flasks (reactors for the bioleaching experiment), volumetric and graduated pipettes, 500 mL plastic bottle, filter paper, cellulose nitrate filter, funnel for filtration, glass filter, falcon tubes, petri dish.

The equipment used is: pH-meter (Hanna), shaking incubator (Shell Lab), balance (Kern, Model-ACS 220-4), spectrophotometer apparatus (Jasco V-530), atomic absorption spectrometer (Varian GTA-110), muffle furnace (Thermolyne), autoclave (AJC, Uniclave-88).

## 3.3. INITIAL CONDITIONS

The iron ore was received from a mine in the Northeast of Portugal.

The first step of experimental work was to determine the distribution size of iron ore particles. With that purpose, 139.5g of iron ore was sieved though 75, 106, 180, 425 μm sieve size (Appendix A, Figure A1.1) to obtain grain size distributions. The obtained results are represented in (Appendix A, Table A1.2). The plot of distribution of particle size represented in the Graphic 1.




Graphic 1. Cumulative curve of particles of iron ore

## 3.4. IRON ORE

The iron ore is the richest rock forming Earth Crust which content is about 5%. The quality of iron ore is mainly estimated by its iron content. The iron ore utilized in this experiment contains 33.20% of iron (Fe), 41.35% silica oxide ($SiO_2$), 3.9% aluminum oxide ($Al_2O_3$), 1.53% phosphorus oxide (V), $P_2O_5$ (0.67% phosphorus, P). This composition was determined using X-ray diffraction. The composition of iron ore is represented in Table 4.

Table 4. Chemical composition of iron ore

| Fe | $SiO_2$ | MgO | $Al_2O_3$ | CaO | $TiO_2$ | $V_2O_5$ | $Cr_2O_3$ | MnO | $P_2O_5$ | P | Sum Oxides | FeO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 33.20 % | 41.35 % | 0.04 % | 3.9% | 0.10 % | <0.84 % | 0.07 % | 0.02% | 0.02 % | 1.53 % | 0.67 % | 97.78 % | 0.63 % |

**pH of iron ore**

For determining the pH, 20.8530 g of iron ore was mixed with 20 mL of distilled water in a 100 mL beaker. pH of the iron ore was measured using pH meter equipment (Figure 5). As a result, pH of iron ore was determined as being 5.89. This value of pH means that iron ore has acidic character.


Figure 4. pH meter (Hanna Instruments)

## 3.5. INOCULUM PREPARATION

The bacteria which was used for bioleaching experiments is *Acidithiobacillus ferrooxidans.*

To prepare the inoculum, 100 mL of bacteria was mixed with 500 mL of medium (composition explained below) and placed into shaking incubator at 28 $^{o}$ C and 120 rpm. The mixture stayed in the incubator and after 1 day a change was observed in the color of mixture, from green to light red, which means the bacteria were grown. The change in colors due to the oxidation of iron (II) to iron (III).

**Preparation of medium for bacteria:**

For phosphorus removal process, it was necessary to prepare the medium DSMZ 882 according to the specifications of the producers of bacteria. For 1L of medium it is necessary to mix 950 mL of solution A and 50 mL of solution B (composition below). After preparation, the pH of both solutions was measured and gradually decreased adding sulphuric acid ($H_2SO_4$) until pH value reached 1.8 for solution A and 1.2 for solution B. After reaching desired pH values, solutions were sterilized in autoclave at different conditions: at 121$^{o}$C during 20 minutes for solution A, and at 112 $^{o}$C during 30 minutes for solution B.

Solution A

To prepare solution A was used 132 mg $(NH_4)_2SO_4$; 53 mg $MgCl_2$ x $6H_2O$; 27 mg $KH_2PO_4$; 147 mg $CaCl_2$ x $2H_2O$. All components were mixed in a volumetric balloon of 1 L and filled with distilled water until dissolving.

Solution B

For solution B, 20 g $FeSO_4$ x $7H_2O$ was dissolved in 50 mL of distilled water.

## 3.6. BIOLEACHING

**Sample preparation for dephosphorization process**

For the experiment of dephosphorization process, into each of 20 Erlenmeyer were placed 15 grams of iron ore, sterilized at 121°C for 15 minutes. After 135 mL of sterilized medium DSMZ 882 and 15 mL of bacteria inoculums, which were incubated during 1 week, in this order, were added to the Erlenmeyer. A blank sample was also prepared: 15 g of soil was placed into an Erlenmeyer and 150 mL of medium for both experiments was added.

For second experiment additionally was prepared second blank with distilled water without medium.

All these samples were placed into a shaking incubator at 28°C and 130 rpm. Initial pH data after adding medium and inoculums were measured as approximately 1.95 (Appendix B, Table B1.1). After adding inoculums to iron ore samples, time counting was started.

Two different experiments were done: in the first experiment the medium DSMZ 882 contained $KH_2PO_4$ and in the second one the medium was prepared without $KH_2PO_4$, because when the medium has this component we are adding phosphorus to the solution. As the main purpose of this work is to remove phosphorus from iron ore using bacteria it is important that the medium does not have this element, because it is easier to the bacteria to use the P in the solution than the P present in the iron ore. The first experiment lasted 42 days and the second 28 days.

**Bioleaching experiment**

After some pre-defined periods of time, several samples were collected in duplicate. The first sample was removed after one day of bioleaching. After removing the sample, this was filtrated, as

can be seen in Figure 5. For the first samples the solution color was slight green, but after 7 days of bioleaching it becomes red that means that iron (II) was oxidized to iron (III) and that the bacteria grow. For each sample pH was measured. After filtration process the obtained iron ore residue was left for drying for 2 days, at atmospheric conditions. . Filtrated solution was placed into 50mL Falcon tubes. All filtrated samples were placed in fridge.

After one day of bioleaching it was observed that the solution and iron ore was not mixing well, maybe because of the high content of iron in the solid. For this reason, rotation of incubator was increased to 160 rpm. The pH of blank was measured every time one sample was removed. The Erlenmeyer with blank was removed only at the end of the experiment.

Obtained pH results of blanks from bioleaching for both experiments are shown in Table5.

Figure 5. Filtration of the samples from bioleaching

Table5. Values of pH of blank solutions (1exp and 2 exp)

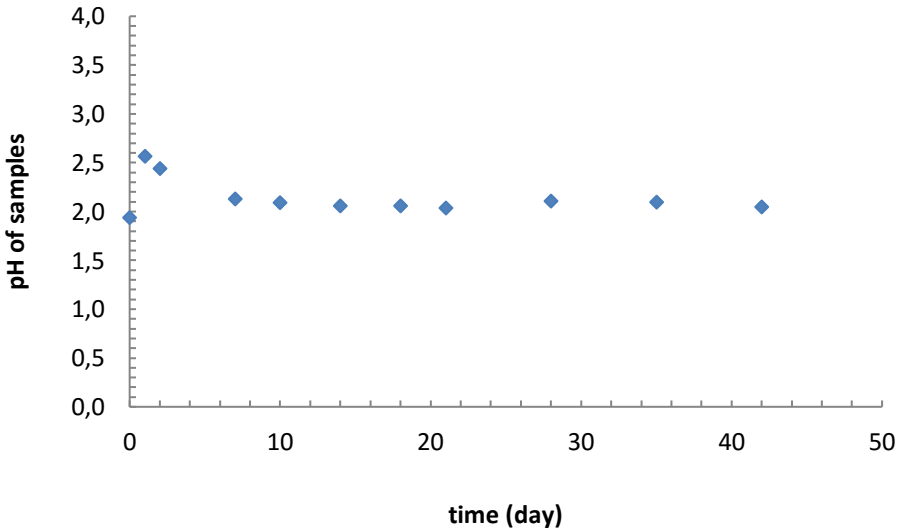
a) values pH of blank of 1st experiment

| Time (day) | pH of blank |
|---|---|
| 1 | 1.95 |
| 2 | 1.90 |
| 7 | 1.94 |
| 10 | 2.19 |
| 14 | 2.81 |
| 18 | 2.80 |
| 21 | 2.05 |
| 28 | 2.09 |
| 38 | 2.05 |
| 42 | 2.02 |

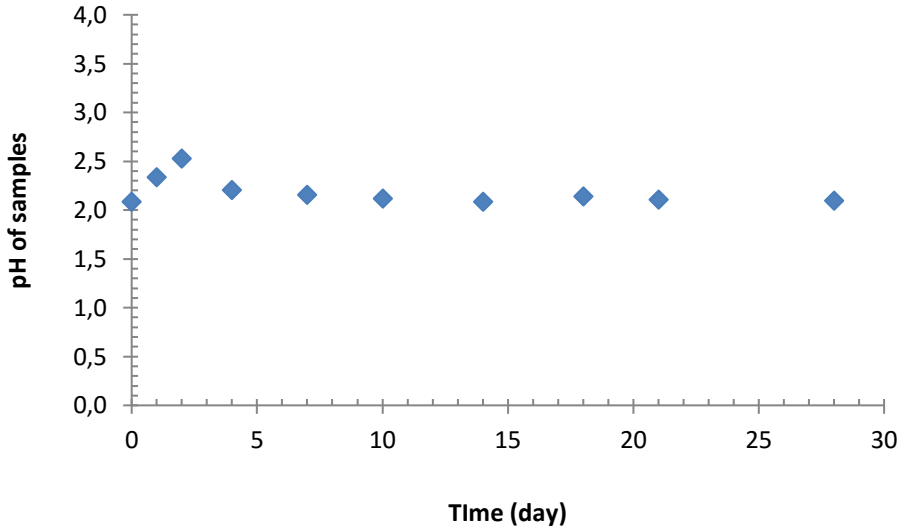
b) values pH of blank of 2nd experiment

| Time (day) | pH of blank |
|---|---|
| 1 | 2.32 |
| 2 | 2.36 |
| 4 | 2.39 |
| 7 | 2.41 |
| 10 | 2.47 |
| 14 | 2.27 |
| 18 | 2.18 |
| 21 | 2.13 |
| 28 | 2.09 |

As can be seen from obtained pH values, the results of pH of first experiment higher, than of second. Graphics below represents pH of samples of bioleaching in a function of time.




Graphic 2. pH in a function of time of bioleaching (1 exp)




Graphic 3. pH in a function of time of bioleaching (2 exp)

## 3.7. PHOSPHORUS ANALYSIS IN SOLUTION

## 3.7.1. ANALYTICAL METHODS

**Determination of phosphorus content by ascorbic acid method**

Phosphorus determination of both experiments by ascorbic acid method was done by UV.Vis analysis using spectrophotometer equipment (Jasco V-530). The spectrophotometer with infrared phototube, was adjusted to 880nm.

For determination of phosphorus by ascorbic acid method it was necessary to prepare a calibration curve.

For this purpose standard phosphorus solutions were prepared with concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/L, from stock phosphate solution with concentration 2.5 mg/L (Appendix C, Table C1.1). These values are used to plot absorbance versus phosphate concentration to give a straight line passing through the origin.

**Preparation of standard solution of phosphorus**

25mL of standard phosphate solution was diluted in 500mL of distilled water. Then, 8; 16; 24; 32; 40 mL of diluted phosphate solution diluted with prepared beforehand diluted medium until

reaching 100 mL. The calibration curve obtained for phosphorus for both experiments is presented in Graphic 4 and Graphic 5.

**Preparation of combined reagent:**

1. $H_2SO_4$ (sulfuric acid) : 70 mL concentrated $H_2SO_4$ was diluted with 500 mL of distilled water.
2. $K(SbO)C_4H_4O_6$ x 1/2 $H_2O$ (potassium antimonyl tartrate ): 0.6858 g of potassium antimonyl tartrate solution diluted with 250 mL of distilled water and was stored in a glass-stoppered bottle.
3. $(NH_4)_6Mo_7O_{24}$ x $4H_2O$ (ammonium molybdate): 10 g of ammonium molybdate solution was diluted 250 mL of distilled water. The prepared solution should be stored in a glass-stoppered bottle.
4. Ascorbic acid: 1.76 g of 0.1M concentrated ascorbic acid dissolved in 100 mL of distilled water.
5. Combined reagent : To obtain 100 mL of combined reagent, mentioned reagents should be mixed in the following proportions : 50 mL 5N $H_2SO_4$, 5 mL potassium antimonyl tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. This mixture should be shake and stand for a few minute until turbidity disappears and was sent to spectrophotometer to analysing absorbance results.

From prepared solution with different concentrations, should be taken 10 mL of solution and add 1.6 mL combined reagent [23].

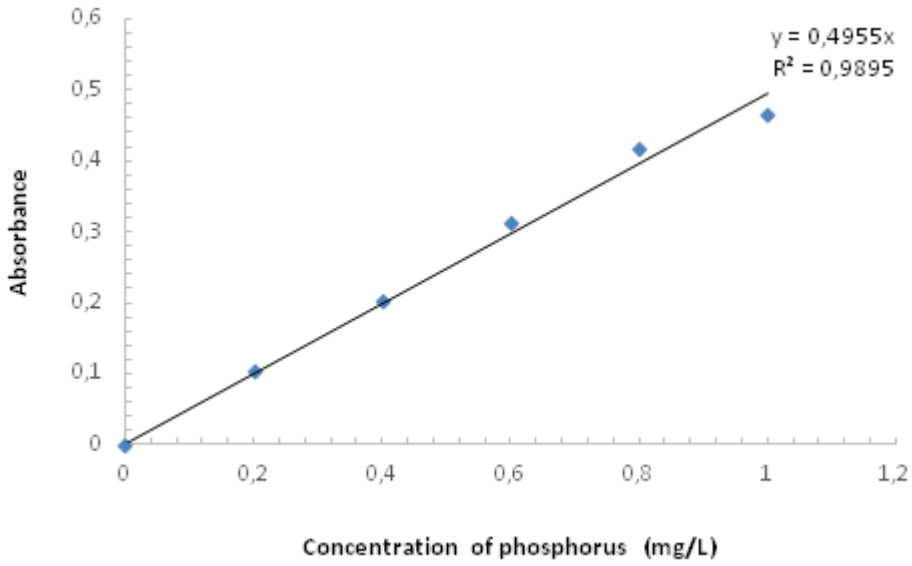
At same time blank solution has to be prepared: 10 mL of medium (diluted 20 times) mixed with 1.6 mL combined reagent and wait 10 minutes (lifetime of combined reagent is 4h).

With adding combined reagent to blank and standard phosphate solution color changed to blue, because of phosphate content. (Figure 6)

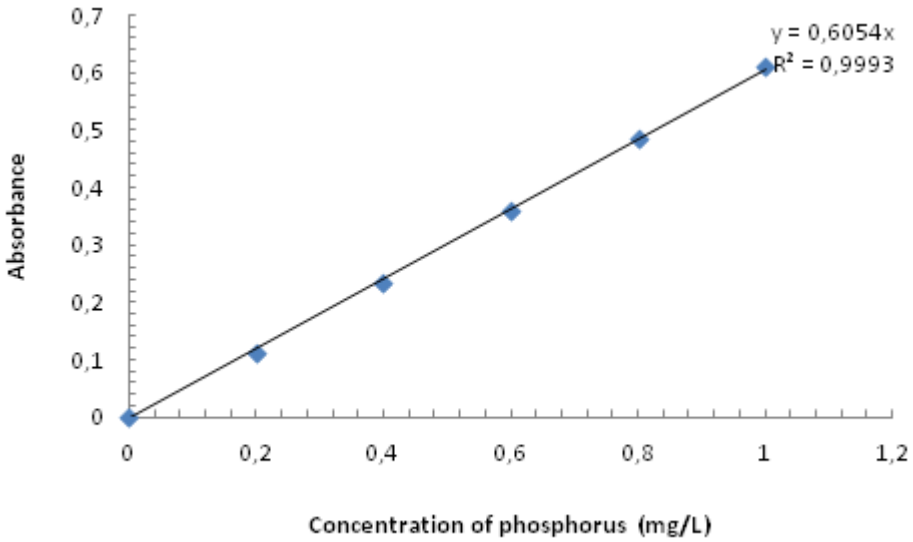
a) Before adding combined reagent b) After adding combined reagent

Figure 6. Blank and standard solutions for phosphorus determination by UV-Vis

Obtained blue color mixture in order to get absorbance value further tested in spectrophotometer apparatus. Absorbance data of standards are presented in Appendix C. The absorbance versus concentration of standards plot graph represented in Graphic 4 and Graphic 5.




Graphic 4. Calibration curve of phosphorus (1 exp)




Graphic 5. Calibration curve of phosphorus (2 exp)

As a result of calibration curves were obtained straight lines of the concentration versus absorbance dependence.

**Determination of phosphorus in the samples**

Filtrated samples from bioleaching were diluted with distilled water in molar ration 1/50 and 1/100 for the first experiment. For the second experiment molar ratio was 1/20 and 1/50.After dilution 10 mL of diluted samples mixed with 1.6mL combined reagent and tested in spectrophotometer apparatus. Obtained values are presented in Appendix C.

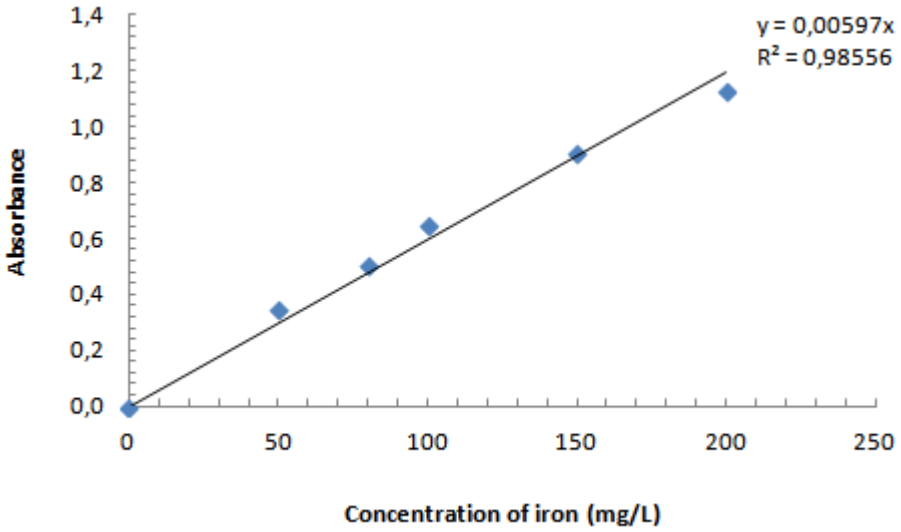
## 3.8. IRON ANALYSIS

### 3.8.1 Atomic absorption

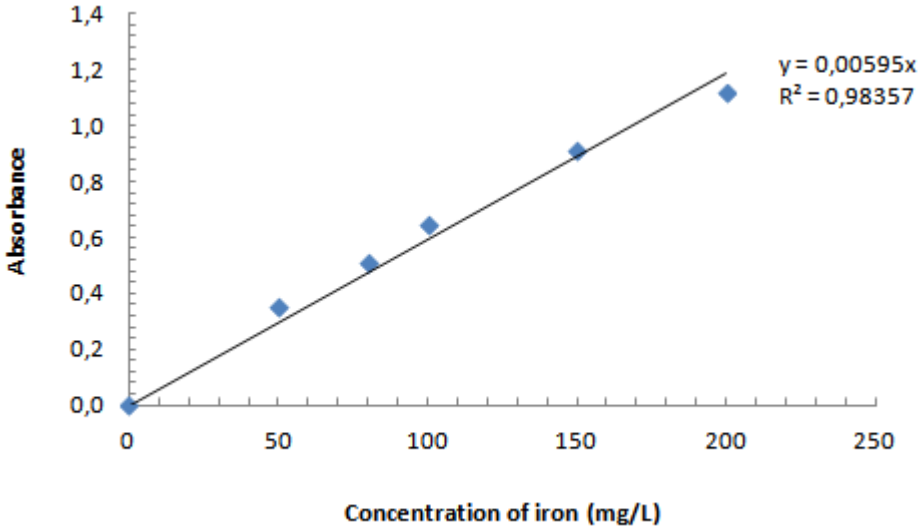
**Preparation of standards**

Atomic absorption is used for determining iron in solution. For this study was prepared 5% $HNO_3$ solution. 50 mL of $HNO_3$ diluted in 1000 mL ultrapure water (Solution A). 50 mL of diluted solution A mixed with 50 mL iron standard solution (1000 mg/L) to obtain solution with 500 ppm concentration. Obtained solution was divided into 5 parts (5 mL, 8 mL, 10 mL, 15 mL, 20 mL) and added 5% $HNO_3$ until 50 mL in order to prepare standard solutions. Separately from samples blank with 5% $HNO_3$ solution was prepared.

Results of calibration curve were represented in Figure 6 and Figure 7.




Graphic 6. Calibration curve of Atomic absorption (1exp)




Graphic 7. Calibration curve of Atomic absorption (2 exp)

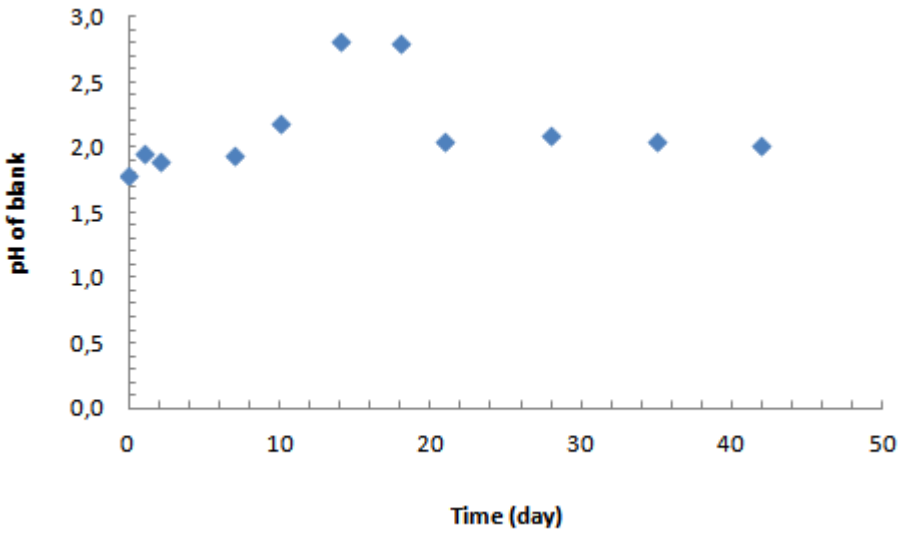
**Preparation samples for atomic absorption**

1 mL from prepared 20 samples was measured and mixed with 5% $HNO_3$ in volumetric balloon until 50mL. Prepared solutions were placed to Atomic Absorption Spectrometer.

# 4. RESULTS AND DISCUSSION

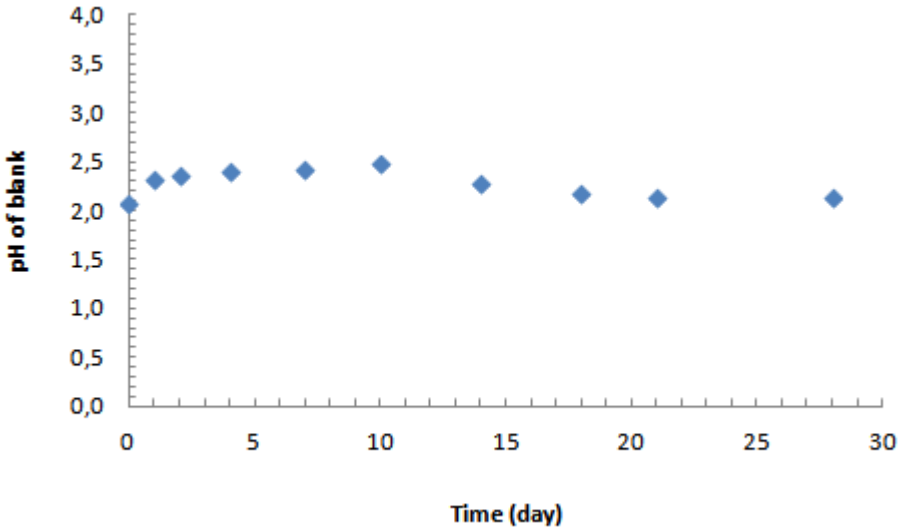
## 4.1 pH analysis

The plot graph of pH of blank values in a function of time presented in Graphic 8 and Graphic 9. As it can be seen from the plot graph, pH values of blank solution in the first days of the experiment slightly increased until reaching the maximum point at 14 and 18 days, then sharply decreased and for the end of the experimental days stabilized.

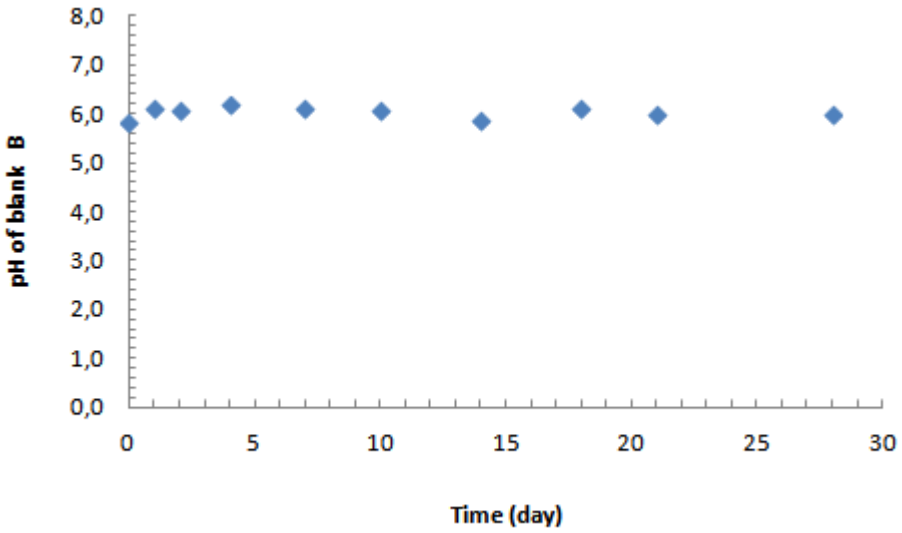



Graphic 8. pH of blank in a function of time (1exp)

According to Graphic 8, it can be seen that two pH values in days of 14 and 18 has higher value, than in another. The reason can be using another pH-meter during measurement.




Graphic 9. pH of blank A in a function of time (2exp)




Graphic 10. pH of blank B in a function of time (2exp)

## 4.2. PHOSPHORUS CONTENT

The removal of phosphorus, in percentage, was determined using the following steps:

The mass of phosphorus in solution was calculated using following formulas:

$$m_{P,medium} = C_{P,solution} * V \qquad (1)$$

Where,

C- Concentration of phosphorus in medium, mg/L

V- Volume of solution, L

To calculate the mass of removed phosphorus, used

$$m_{P,removed} = (C * V) - m_{P,medium} \quad (2)$$

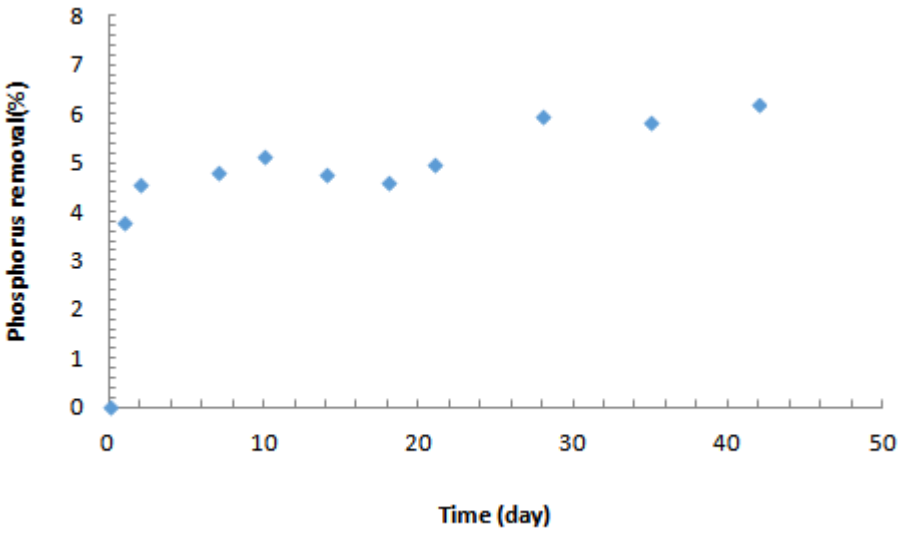
Where,

C- Concentration of diluted samples mg/L

V- Volume of solution, L

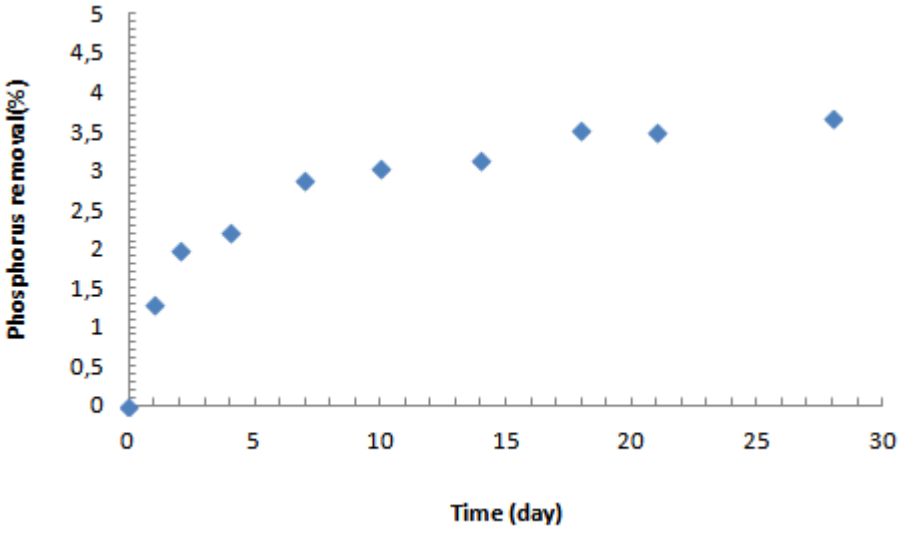
$$\%P_{removed} = \frac{m_{P,removed}}{m_{P,iron\ ore}} * 100 \quad (3)$$

Phosphorus removal in a function of time was presented in Graphic 11 and Graphic 12.




Graphic 11. Phosphorus removal as a function of time (1 exp)

As it can be seen from the Graphic 11, for the first experiment the percentage of phosphorus removal increases in the first 10 days, then there is a slightly fluctuation until day 28, and then after 28 days it tends to a level of constant value.




Graphic 12. Phosphorus removal as a function of time (2 exp)

As can be seen from Graphic 12, the percentage of phosphorus removal of second experiment increases with time till day 18 and after, like the first experiment, it tends to a level of constant value.

## 4.3 IRON CONTENT

The results of iron content (%) in iron ore in a function of time for both experiments are presented in Table 6 and Table 7.

Table 6. Iron content percentage in first experiment

| Time (day) | Iron % |
|---|---|
| 1 | -0,199 |
| 4 | -0,410 |
| 7 | -0,766 |
| 10 | -2,050 |
| 14 | -3,255 |
| 18 | -3,318 |
| 21 | -3,435 |
| 28 | -3,436 |
| 35 | -3,405 |
| 42 | -3,550 |

Table 7. Iron content percentage in second experiment

| Time (day) | Iron % |
|---|---|
| 1 | 0,858 |
| 2 | 1,205 |
| 4 | 1,171 |
| 7 | -0,494 |
| 10 | -2,282 |
| 14 | -2,239 |
| 18 | -2,225 |
| 21 | -2,419 |

The small negative percentage of iron indicates that bacteria did not dissolve iron in both experiments.

# 5. CONCLUSIONS AND FUTURE WORK

## 5.1 CONCLUSIONS

For the purpose of dephosphorization of iron ore from Northeast of Portugal, two different experiments of bioleaching were carried out. For both experiments the microorganism used was *Acidithiobacillus ferrooxidans*, which was supplied by DSMZ collection, the temperature was 28°C, the agitation 160 rpm, ratio solid/solution was 1/10. The medium in the first experiment had $KH_2PO_4$ in its composition while the second experiment didn´t have this compound. Also, the first experiment lasted 42 days and the second 28 days. From the results obtained, it was observed that for first experiment the yield of phosphorus removal was 6.19 wt%, after 42 days, and for second experiment was 3.66 wt%, after 28 days.

These values obtained in the preliminary assays are relatively low, when the purpose of the work is to obtain an iron ore with a percentage of phosphorus less than 0.08%. The reason of these small percentage of phosphorus removal may be related with the mineral composition of the iron ore analyzed. It was not possible to determine in which mineral phase phosphorus is. It is possible that phosphorus is integrated in the crystal lattice of iron ore oxides and that it is more difficult to remove it. Localization of phosphorus may be such that it is hard to the microorganisms to access this element. It is possible that phosphorus is associated to goethite and it turns to be more difficult to remove it.

## 5.2 FUTURE WORK

To improve process of bioleaching, in first place it is important to study the growth of bacteria. It is important to monitor the bacteria growth, because bacteria's growth is an important factor to dephosphorization process.

The work that was done is a preliminary study to the dephosphorization of the iron ore supplied by the mine in the Northeast of Portugal. There are some relevant factors associated to the bioleaching processes that should be studied: grain size of the particles of the iron ore (here almost 90% of the particles have size less than 75 (μm), initial pH of the bioleaching solution, inoculum concentration, temperature, reaction time, solid/liquid ratio.

It would also be interesting to analyze the final residue (iron ore after the bioleaching) to verify the remaining percentage of phosphorus. We tried to digest the residue using aqua regia but so far it did not give satisfactory results, so it is recommended to try another solution for digestion method.

## REFERENCES


[1] http://www.gsa.org.au

[2] Blaney, Lee, "Magnetite (Fe3O4): Properties, Synthesis, and Applications" (2007). *Volume 15 - 2007.* Paper 5.

[3] S. S. Shinde, R. A. Bansode, C. H. Bhosale, and K. Y. Rajpure. Physical properties of hematite _-Fe2O3 thin films: application to photoelectrochemical solar cells.Journal of Semiconductors, 2011, 32(1).

[4] B. Roger , JR. Herbert, Properties of goethite and jarosite precipitated from acidic groundwater, Dalarna, Sweden, Clays and Clay Minerals, 45( 2),1997, 261-273.

[5] Y. Hexiong, L. Ren , D. T. Robert , C. Gelu , Goethite, α-FeO(OH), from single-crystal data, Acta Crystallographica Section E, 62 ,(2006), 250–252.

[6] Erik Jonsson, Valentin R. Troll1, Karin Ho¨gdahl, Chris Harris, Franz Weis, Katarina P. Nilsson

& Alasdair Skelton. Magmatic origin of giant 'Kiruna-type'apatite-iron-oxide ores in CentralSweden. Scientificreports, 2013, 3: 1644/

[8] Antônio Clareti Pereira, RísiaMagriotisPapini. REM: R. Esc. Minas, Ouro Preto. Processes for phosphorus removal from iron ore - a review**.** Mining Mineração, 2015, 68(3), 331-335.

[7] Bertil I. Palsson, Olof Martinsson, Christina Wanhainer and Andreas Fredrikson. Unlocking Rare Earth Elements From European Apatite-Iron Ores. ERES 2014: 1st European Rare Earth Resources Conference, Milos, 2014.

[9] FathiHabashi. 2005. A short history of hydrometallurgy. Hydrometallurgy, **79**: 15-22.

[10] Jaime Wisnaik. Phosphorus from discovery to commodity. Indian Journal of Chemical Technology, 2005, 12: 108-122.

[11] A.E. Johnson & I. Steen. Understanding Phosphorus and its use in Agriculture. European Fertilizer Manufacturers Association, Pp. 1-38. [12] Chime T. O., Menkiti M. C., Onukwuli O. D. 2011. Biodephosphorization of iron Ore using *Acidothiobacillusferrooxidans*. NewYork Science Journal, **4(1)**: 1-6.

[13] Jin, Y. et al. Removal of phosphorus from iron ores by chemical leaching. Journal of Central South University of Technology, v. 13, n. 6, p. 673-677, 2006.

[14] Ranjbar, M. Dephosphorization of Iranian iron oxide fines by flotation. Erzmetall, v. 55, n. 11, p. 612–620, 2002.

[15] Watling, H. R. The bioleaching of sulphide minerals with emphasis on copper sulphides – a review. Hydrometallurgy, v. 84, n. 1-2, p. 81-108, 2006.

[16] Mishra D, Kim D, Ahn J, Rhee Y. 2005. Bioleaching: A microbial process of metal recovery; A review. Metals and Materials International, **11 (3)**: 249-256.

[17] Klaus Bosecker. 1997. Bioleaching: metal solubilisation by microorganisms. Federation of European Microbiological Sciences Microbiology Reviews, **20**: 591-604.

[18] Lacey, D. T. and Lawson, F. 1970. Kinetics of the liquid phase oxidation of acid ferrous sulphate by the bacterium, *Thiobacillusferrooxidans*. Biotechnology Bioengineering, **12**: 29-59.

[19] G. J. Olson · J. A. Brierley · C. L. Brierley. Bioleaching review part B:Progress in bioleaching: applications of microbial processes by the minerals industries. ApplMicrobiolBiotechnol (2003) 63:249–257.

[20] S. K.Masud Hossain, Manas Das, S. H. Ibrahim, K. M.MSheriffa Begum and N Anantharaman. 2004. Studies on Biohydrometallurgical leaching of iron sulphide ore using *Thiobacillusferroxidans*.

[21] Seifelnassr, A. A. S. and Abouzeid, A. M. 2013. Exploitation of bacterial activities in mineral industry and environmental preservation: An overview. Journal of Mining, Hindawipublishingcorporation.

[22] Environmental Biotechnology: Theory and Application**.** By Gareth M. Evans, Judith C. Furlong.

[23] Standard Methods for the examination of water and wastewater, Edited by Lenor S. Clesceri et al. American Public Health Association, 1998

# Appendix A

## Materials


Figure A.1.1 Sieves

Table A1.2. Obtained grain size data for cumulative curve

| Weight (g) | Size (µm) | Cumulative curve (%) |
|---|---|---|
| 25.11 | 75 | 18.6 |
| 41.71 | 106 | 29.9 |
| 97.51 | 180 | 69.9 |
| 138.24 | 425 | 99.1 |

# Appendix B

## Appendix B1 Phosphorous analysis in solution

Table B1.1 Weight data and initial pH of samples

a) 1st experiment

| Sample | weight of ore (g) | pH |
|---|---|---|
| 1 | 15.0062 | 1.98 |
| 2 | 15.0063 | 1.95 |
| 3 | 15.0003 | 1.94 |
| 4 | 15.0030 | 1.92 |
| 5 | 15.0070 | 1.91 |
| 6 | 15.0023 | 1.94 |
| 7 | 15.0026 | 1.95 |
| 8 | 15.0035 | 1.91 |
| 9 | 15.0008 | 1.93 |
| 10 | 15.0031 | 1.90 |
| 11 | 15.0020 | 1.94 |
| 12 | 15.0011 | 1.92 |
| 13 | 15.0072 | 1.91 |
| 14 | 15.0022 | 1.93 |
| 15 | 15.0039 | 1.94 |
| 16 | 15.0041 | 1.96 |
| 17 | 15.0074 | 1.91 |
| 18 | 15.0023 | 1.93 |
| 19 | 15.0036 | 1.92 |
| 20 | 15.0133 | 1.94 |

b) 2nd experiment

| Sample | weight of ore (g) | pH |
|---|---|---|
| 1 | 15.0003 | 2.12 |
| 2 | 15.0124 | 2.10 |
| 3 | 15.0151 | 2.11 |
| 4 | 15.0030 | 2.10 |
| 5 | 15.0040 | 2.12 |
| 6 | 15.0018 | 2.10 |
| 7 | 15.0032 | 2.12 |
| 8 | 15.0017 | 2.15 |
| 9 | 15.0135 | 2.09 |
| 10 | 15.0009 | 2.09 |
| 11 | 15.0071 | 2.07 |
| 12 | 15.0055 | 2.08 |
| 13 | 15.0072 | 2.08 |
| 14 | 15.0036 | 2.09 |
| 15 | 15.0077 | 2.10 |
| 16 | 15.0159 | 2.07 |
| 17 | 15.0191 | 2.09 |
| 18 | 15.0184 | 2.07 |
| 19 | 15.0040 | 2.08 |
| 20 | 15.0062 | 2.11 |

# Appendix C Analytical methods

Table C1.1 Absorbance data for calibration curve of phosphorus (1exp)

| Concentration | Absorbance |
|---|---|
| Blank | 0.0026 |
| 0.2 | 0.1043 |
| 0.4 | 0.2020 |
| 0.6 | 0.3137 |
| 0.8 | 0.4187 |
| 1.0 | 0.4652 |

Table C1.2 Absorbance values of diluted samples in distilled water (1exp)

| Time(day) | Samples | Dilution | Absorbance | Concentration (mg/L) |
|---|---|---|---|---|
| 1 | 1 | 1/50 | 0.3018 | 30.454 |
| | 2 | 1/50 | 0.3247 | 32.765 |
| 2 | 3 | 1/50 | 0.3640 | 36.731 |
| | 4 | 1/50 | 0.3632 | 36.650 |
| 7 | 5 | 1/50 | 0.3665 | 36.983 |
| | 6 | 1/50 | 0.3934 | 39.697 |
| 10 | 7 | 1/50 | 0.4113 | 41.504 |
| | 8 | 1/50 | 0.3938 | 39.738 |
| 14 | 9 | 1/50 | 0.4292 | 43.310 |
| | 10 | 1/50 | 0.3273 | 33.027 |
| 18 | 11 | 1/50 | 0.3731 | 37.649 |
| | 12 | 1/50 | 0.3624 | 36.569 |
| 21 | 13 | 1/50 | 0.3578 | 36.105 |
| | 14 | 1/50 | 0.4236 | 42.745 |
| 28 | 15 | 1/50 | 0.4488 | 45.288 |
| | 16 | 1/50 | 0.4640 | 46.821 |
| 35 | 17 | 1/100 | 0.2008 | 40.525 |
| | 18 | 1/100 | 0.2476 | 49.970 |
| 42 | 19 | 1/100 | 0.2278 | 45.974 |
| | 20 | 1/100 | 0.2436 | 49.162 |

Table C1.3 Absorbance data for calibration curve of phosphorus (2 exp)

| Concentration | Absorbance |
|---|---|
| 0,2 | 0,1111 |
| 0,4 | 0,2353 |
| 0,6 | 0,3605 |
| 0,8 | 0,4859 |
| 1 | 0,6105 |

Table C1.4 Absorbance values of diluted samples in distilled water (2 exp)

| Time (day) | Samples | Dilution | Absorbance | Concentration(mg/L) |
|---|---|---|---|---|
| 1 | 1 | 1/50 | 0.1154 | 9.531 |
| | 2 | 1/20 | 0.2354 | 7.777 |
| 2 | 3 | 1/20 | 0.3799 | 12.550 |
| | 4 | 1/20 | 0.4257 | 14.063 |
| 4 | 5 | 1/20 | 0.4501 | 14.870 |
| | 6 | 1/20 | 0.4458 | 14.727 |
| 7 | 7 | 1/50 | 0.2442 | 20.168 |
| | 8 | 1/20 | 0.5556 | 18.355 |
| 10 | 9 | 1/50 | 0.2417 | 19.962 |
| | 10 | 1/50 | 0.2526 | 20.862 |
| 14 | 11 | 1/20 | 0.6078 | 20.079 |
| | 12 | 1/50 | 0.2668 | 22.035 |
| 18 | 13 | 1/50 | 0.2878 | 23.769 |
| | 14 | 1/50 | 0.2858 | 23.604 |
| 21 | 15 | 1/50 | 0.2797 | 23.100 |
| | 16 | 1/50 | 0.2879 | 23.778 |
| 28 | 17 | 1/50 | 0.2942 | 24.298 |
| | 18 | 1/50 | 0.3016 | 24.909 |

# Appendix D Phosphorus Content

Table D1.1. Percentage of phosphorus removal (1exp)

| Time (day) | %P removed |
|---|---|
| 1 | 3,80 |
| 2 | 4,56 |
| 7 | 4,80 |
| 10 | 5,14 |
| 14 | 4,78 |
| 18 | 4,62 |
| 21 | 4,96 |
| 28 | 5,95 |
| 35 | 5,83 |
| 42 | 6,18 |

Table D 1.2 Percentage of phosphorus removal (2exp)

| Time (day) | %P removed |
|---|---|
| 1 | 1,29 |
| 2 | 1,99 |
| 4 | 2,20 |
| 7 | 2,87 |
| 10 | 3,05 |
| 14 | 3,14 |
| 18 | 3,53 |
| 21 | 3,49 |
| 28 | 3,66 |

# Appendix E

Table E 1.1 Values of pH samples in function of time

a) 1st experiment

| Time (day) | pH |
|---|---|
| 1 | 2.52 |
| | 2.62 |
| 2 | 2.44 |
| | 2.43 |
| 7 | 2.13 |
| | 2.12 |
| 10 | 2.09 |
| | 2.08 |
| 14 | 2.07 |
| | 2.05 |
| 18 | 2.05 |
| | 2.06 |
| 21 | 2.02 |
| | 2.05 |
| 28 | 2.10 |
| | 2.11 |
| 35 | 2.10 |
| | 2.08 |
| 42 | 2.05 |
| | 2.04 |

b) 2nd experiment

| Time (day) | pH |
|---|---|
| 1 | 2.35 |
| | 2.33 |
| 2 | 2.53 |
| | 2.52 |
| 4 | 2.21 |
| | 2.21 |
| 7 | 2.15 |
| | 2.16 |
| 10 | 2.12 |
| | 2.11 |
| 14 | 2.09 |
| | 2.08 |
| 18 | 2.14 |
| | 2.13 |
| 21 | 2.11 |
| | 2.10 |
| 28 | 2.10 |
| | 2.08 |